

Etude expérimentale sur la pénétration intra-oculaire de l' ammoniacque
[Experimental study on the intra-ocular penetration of ammonium hydroxide]

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ABSTRACT

[An English Abstract is provided in the French original.]

INTRODUCTION

This experimental study was undertaken following the results of the authors' prospective clinical study of base chemical ocular burns in Martinique [1,2]. This study reported a significant frequency of ocular burns with Alkali® (ammonium hydroxide 15.3%; pH 12.8) and seemed to indicate that ocular lavage done before a delay of several minutes might prevent severe ocular burns. Classical descriptions from the literature seemed to contradict these findings, stating that the severity of ocular base chemical burns can be explained by the capacity of base chemicals to very rapidly penetrate and cross through the cornea, and by their capacity to injure the deep ocular tissues [3-5].

Some experimental studies of ammonium hydroxide gave an idea of the rapidity of this penetration and of the relationship which exists between this penetration and the severity of the ocular burns. In this fashion, Siegrist [6] experimentally found ammonium hydroxide in the aqueous humor 30 seconds after it was instilled on the cornea, but did not do measurements of its concentration, nor of pH. Grant [7] demonstrated an increase in pH of the aqueous humor to 9.4 30 seconds after exposing rabbit eyes to a 28.5% ammonium hydroxide solution for 10 seconds. He also demonstrated that an aqueous humor pH greater than 11 is necessary to result in severe lesions of the cornea, iris, and crystalline lens.

More recently, Paterson [8] demonstrated an increase of pH to greater than 11 one minute after exposing rabbit eyes to 100 Φ l of a 28.5% ammonium hydroxide solution. He also showed the absence of significant pH changes following ocular lavage with normal saline begun at 2 to 3 minutes after the burn. Moreover, he found a return to a normal pH 30 minutes to 3 hours after the burn.

From the above, the question can be posed: does the delay from the clinical study correspond to the time for penetration of a significant quantity of ammonium hydroxide and for it to cross through the cornea? Alternatively stated, does the pH of the anterior chamber reflect in a strict sense the number of moles of ammonium hydroxide which have passed through the cornea (assuming that the cornea is a semi-permeable membrane), or is it a reflection of a new chemical equilibrium resulting from interactions between the base chemical and the ocular tissues (in which case the cornea would constitute a biological substrate)? The following *in vivo* study was conceived to answer the above question.

MATERIAL AND METHODS

This experimentation was carried out in 23 eyes of New Zealand white rabbits weighing 2 to 2.2 kg. Each rabbit was anesthetized by intramuscular injection of 1 ml of a solution constituted of 1 ml = 2 mg of flunitrazepam (Narcosep®) and 1 ml of normal saline; then 30 minutes later by subcutaneous injection of 7 ml of a solution of 6 g/100 ml of sodium pentobarbital diluted in 50 ml of normal saline, divided into 4 injection sites. The rabbits were anesthetized 15 minutes later.

The eyelids were maintained open with a Barraquer Colibri 15 mm blepharostat. The rabbit eyes were topically anesthetized with Novésine® (oxybuprocaine), then the conjunctival cul-de-sac was cleared of tears and excess Novésine® with an ophthalmic pledget. The probe of the pH meter (1.3 mm in diameter) was placed in the anterior chamber by catheterization with an 18 gauge trocar (1.2 mm in diameter). The trocar was introduced at the level of the perilimbic cornea, with the bevel directed inferiorly. This catheterization technique assures the minimum loss of aqueous humor.

Next, the probe of the pH meter was introduced into the anterior chamber through this channel, bevel oriented inferiorly to prevent any loss of aqueous humor and to avoid producing any lesions of the iris; then the bevel was re-directed superiorly and its tip is placed in the center of the anterior chamber. The operator maintained it in place during the experimentation. The probe utilized was an MI 413 B7 , no. 699-11-001, manufactured at Bedford [MA] (United States of America) by MICROELECTRODES, INC. The pH meter was a pH M 240 Radiometer®, no. 658R006N005, manufactured in Lyon (France) by Radiometer Analytical, S.A.

The pH of the aqueous humor was then recorded before any manipulation and the stability was ensured by several measurements. Next, another operator placed a 7 mm diameter Hoffer optic marker in the eye on the center of the cornea. This played the role of a micro-reservoir with an open bottom. With a micro-pipette calibrated in 0.01 ml, a solution of 15.3% ammonium hydroxide was placed in the interior of the optic marker.

The ammonium hydroxide solution was maintained in the cavity of the optic marker for 1 minute, and then the marker was removed. The choice of this method was dictated by the obligation to compare this experimental study with the clinical study. It was thus necessary to utilize the ammonium hydroxide at a concentration of 15.3%. It was also necessary to have volumes which were similar to those seen clinically. The palpebral reflex in effect causes a sweeping action on the corneal surface such that only 0.2 ml actually remains in contact with the eye. For this reason, a volume of 0.1 ml was chosen and maintained on the corneal surface with the 7 mm diameter optic marker, which is to say on 38.5 mm² of the corneal surface (or about half the surface area). For comparison, it is known that in cases of total immersion the density of the liquid which is in contact with the skin is 0.02 ml/cm². It thus seems that except for the dilution effect due to tears, the experimental condition approaches the clinical condition.

The pH measurements were repeated throughout the experimental trials at intervals of 10 seconds for the trials for 30 minutes and at 5 seconds for the other trials. In 5 eyes, punctures of the anterior chamber with a 30 gauge needle on an insulin syringe were done at 1, 3, 5, 10, and 30 minutes (*fig. 1*). In the other 18 eyes, these anterior chamber punctures were done after external ocular lavage. Then measurements of the ammonium hydroxide concentration in the aqueous humor were made.

These ammonium hydroxide concentration measurements were done with a Synchron CX® system from Beckman Laboratory (Fullerton, California, USA). This consists of quantitative measurements according to a final point technique. In view of the high levels of ammonium hydroxide which were found, a dilution of each sample by one-hundred was necessary to complete the measurements. This is an enzymatic method which measures the total of ammonium hydroxide present; that is, the concentration of the ammonium ion NH₄⁺ and the concentration of ammonium hydroxide itself (NH₄OH). This measurement method has analytical limits of 5 to 1,000 Φmol/l.

The quasi-totality of the measurements done were less than this concentration. These measurements were obtained by diluting the aqueous humor samples by 1/10 or 1/100 with de-ionized water. In the absence of dilution, the margin of error was 5 Φ mol.

The reliability of the measurements was verified by testing 5 samples with exactly known concentrations of ammonium hydroxide and ammonium ion. To facilitate the assessment, a total concentration of 40.147 mmol/l was chosen. Thus, each measurement necessitated a 1/100 dilution. The characteristics of each sample as well as the measurement results are shown in *Table I*. There was a mean under-estimation of the total ammonium hydroxide concentration of 7.7046 mmol/l. From this concentration, it is possible to calculate the theoretical pH (pH th. in the figures) by the formula: $\text{pH} = 7 + 2 \text{ pKa} + 2 \log [\text{NH}_4\text{OH}]$, with $\text{pKa} = 9.2$ for ammonium hydroxide.

RESULTS

The results of each experimental trial are shown in the figures in the form of the pH graph as a function of time. The time recording of the experimental trials begins with the application of the ammonium hydroxide to the eye. On these graphs is displayed the concentration of ammonium hydroxide in the aqueous humor in millimoles, measured from each sampling, as well as the theoretical pH (pH th. in the figures).

The pH before experimentation was variable from one trial to another. The group of graphs demonstrated an increase of the pH in the aqueous humor from 1 to 3 minutes after the ammonium hydroxide application. The maximal pH peak was obtained 5 to 6 minutes after the beginning of the ammonium hydroxide application and reached a maximum of 10. All the graphs were comparable with a plateau between two increases of the pH curve. This form of the curve is quite clear for the 5 trials conducted at 5 minutes. In this manner, for example, the trial described in *figure 2* successively shows an initial pH increase, a plateau from 2.20 minutes to 3.30 minutes, then a second increase (from 3.30 minutes to 3.30 minutes), and finally a second plateau.

For the graphs of short duration, only the first plateau is shown. For the graphs of long duration, the plateaus seem much smaller because the time frame is longer. For the graphs of 10 and 30 minutes duration (*fig. 3 and 4*) the pH evolves following the peak in the form of an exponential decrease. However, even after 30 minutes the pH did not return to initial values. In contrast, the graphs of 3 trials conducted for 30 minutes reveal low concentrations of ammonium hydroxide (1 to 5 millimoles). Finally, on all the graphs, the measured pH was always lower than the theoretical pH. It was also possible to calculate the level of penetration of ammonium hydroxide.

In effect, at 15.3%, in 100 grams of solution there are 15.3 g of ammonium hydroxide and 84.7 g of water. Knowing the density of ammonium hydroxide B 0.943 B it is possible to calculate the number of moles placed on the cornea in 0.01 ml of a 15.3% solution. The mass of ammonium hydroxide present in 0.01 ml of this solution corresponds to: $0.00943 \times 0.153 = 0.0014427$ g. The molecular mass of ammonium hydroxide is 17; the number of moles present in 0.01 ml of the solution is thus: $0.0014427/17 = 84.9 \Phi\text{mol}$. In other words, the 0.01 ml of solution placed on the cornea had an ammonium hydroxide concentration of 8.49 mol/l. From these data it is possible to calculate the level of ammonium hydroxide penetration, as a ratio of the number of moles retrieved from the aqueous humor and the number of moles of ammonium hydroxide placed on the cornea. If the highest concentration is chosen B 75.1 millimol/l, by approximation considering this the maximum volume, then the number of moles in the aqueous humor would be 7.51 Φ mol. At the

maximum, it seems that $7.51/84.9 \text{ } \Phi\text{mol} = 11\%$ of the ammonium hydroxide placed is retrieved from the aqueous humor.

DISCUSSION

The interpretation of this study should take into account possible biases. Ammonium hydroxide is a volatile product and an exact reproducibility of the number of moles placed on the cornea is in fact impossible. Measurements of anterior chamber pH before ammonium hydroxide application were different from one trial to another. This difference might be explained by poor calibration of the pH probe. This calibration was done before each trial and this probability remains low. In any case, the maximum of pH variations due to poor calibration should be 0.5, more or less. Regardless, the measurements of relative pH variations remains valid.

The position of the probe in the anterior chamber may cause some variations in the pH measurements, but significant variations are only found when the probe is placed behind the iris. Throughout each trial, an operator constantly verified the position of the probe, but it was noted that control is difficult during lavage. Thus, the difference between the measured pH and the theoretical pH can be explained in part by the above factors.

Control testing (see AMethods@) demonstrated a mean under-estimation of the ammonium hydroxide concentration of 7.7046 mmol/l . A final possible bias in the measurement of the ammonium hydroxide concentration is measurement of ammonium hydroxide normally present in the aqueous humor from metabolism of amino acids. Measurements of aqueous humor ammonium hydroxide concentrations were not found in the literature. If it is similar to that in venous blood where ammonium hydroxide is found at a level of $18 \text{ } \Phi\text{mol/l}$, in 0.1 ml of aqueous humor there would be $0.0018 \text{ } \Phi\text{mol}$, a negligible quantity.

The rapidity of the penetration of ammonium hydroxide was well demonstrated by the beginning of an increase in the aqueous humor pH 1 to 3 minutes after application. These data are comparable to those from the literature [6-8]. The trials at 30 minutes translated into graphs directly comparable to those of Paterson [8], with a peak at about 5 minutes followed by a very progressive exponential decrease. It must be noted that after this delay of 30 minutes, there always had not been a return to physiologic pH, with measured pH about 9. On this basis, Paterson affirmed the lack of efficacy of ocular lavage. Measurements of the concentration of ammonium hydroxide at 30 minutes were low in the current study, which supports a supplemental argument that the interest of external ocular lavage is no doubt limited after 30 minutes. Moreover, this experimental argument is concordant with the data from the authors' human clinical study [1,2]. Nevertheless, it should be remembered that the experimental data only involve the anterior chamber and only indirectly represent the corneal problem.

Finally, this study placed in evidence the absence of correlation between the measured pH and the theoretical pH, calculated from the ammonium hydroxide concentration by the formula:

$$\text{pH} = 7 + 2 \text{ pKa} + 2 \log [\text{NH}_3]$$

The theoretical pH derived from this formula was always higher than the measured pH. This is an inherent bias in the measurement of the ammonium hydroxide concentration. This theoretical formula is in fact only valuable if the ammonium hydroxide is not reactive. However, there are

chemical reactions. Analysis of the pH graphs which present two increases followed by a plateau confirms this hypothesis. Each plateau corresponds to a chemical equilibrium: the reaction of ammonium hydroxide (NH₄OH) with the acids (AH) in the aqueous humor, that is to say amino acids, or aqueous humor proteins. These chemical reactions can be schematized as follows:



At the beginning of the increase, there was thus some ammonium hydroxide and some acid. Then, at the completion of the increase, when all the acid was consumed, there remained ammonium ion, the conjugated base of the acid (A⁻) and some water. Another chemical equilibrium was then established. Then some further amount of ammonium hydroxide diffused in and a second chemical reaction commenced following the same reaction with another acid.

At the equivalence point corresponding to identical concentrations of the base and the acid (milieu during the pH increase) the pH = 2 (pK NH₄OH/NH₄⁺) + pK (AH/A⁻). From this can then be deduced the pK of the involved acid. The slope of the pH curve depends on the diffusion of the ammonium hydroxide. Thus, the main experimental data have been taken into account following the theory of Blomet [10] which explains the hazards of a chemical product for biological tissues.

In effect, from the experimental data it is possible to calculate the density of the proteins consumed by the ammonium hydroxide which penetrates into the aqueous humor. The concentration of NH₄⁺ ions in the aqueous humor is a result of the reaction of ammonium hydroxide (NH₄OH) with acids (AH) in the aqueous humor, and thus is equal to the concentration of acids (AH) consumed by this reaction. Therefore, there are two unknowns, but two equations:

$$[\text{NH}_4\text{OH}] + [\text{NH}_4^+] = \text{measured concentration} \quad (1)$$

$$\text{pH measured} = \text{pK} + \log \text{NH}_4\text{OH}/\text{NH}_4^+ \quad (2)$$

It is therefore possible to calculate the density of the proteins consumed by the action of ammonium hydroxide. For this, it is then necessary to know the speed of penetration and the total volume of ammonium hydroxide which can be calculated mathematically from the slope of the curve. If an identical experiment was repeated with another base which penetrates into the aqueous humor, it would then be possible to calculate the density of the proteins consumed by this base. It would remain to compare these two densities and to correlate with the pK of each base. In effect, according to the theory of Blomet [10] the relative hazard of a base for biological tissues is due to its pK.

According to this theory, the chemical reactions themselves are not the hazard, but rather this follows a scale of energetic potential. In other words, a base may react with an acid whose pK is much lower than its own, and then go on to react with acids with a pK which is greater than its own. Then, if after this reaction there still remains some base, it can react with another acid whose pK is lesser, and so on. This was found in analyzing the pH curves from the present study. In this fashion, comparison of the density of the proteins consumed by one base in comparison with another would permit the verification of this theory whose implications are major as the hazard for biological tissues of a chemical product X could then be correlated with its pK. This indication could then be

represented in the packaging of chemical products, and physicians could then know its hazard potential. The actual risk is itself due to the number of moles (in other word, the concentration and thus the pH) deposited on human tissue since the chain reactions can only continue if the base (in the present case) is not consumed by the initial reactions.

CONCLUSION

The present study confirms the rapid penetration of ammonium hydroxide: 1 to 3 minutes to cross the cornea. However, at the maximum only about 10% of the ammonium hydroxide placed on the cornea appears to be found in the aqueous humor. This was the first study which combined anterior chamber pH measurement with measurement of anterior chamber ammonium hydroxide concentrations. This has permitted the demonstration of the possibility of *in vivo* measurement of the density of the proteins consumed by a base which has penetrated into the aqueous humor. This suggests some interesting research directions. In effect, if this study can be replicated for other bases with different $pK=s$, it might then be possible to correlate the pK of a base with the density of the proteins consumed in the eye; otherwise stated, to evaluate the hazard potential of a base for biological tissues.

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REFERENCES

[See French Original.]

TABLE I

Validity limits of the measurements of the anterior chamber concentration of ammonium hydroxide: there is a mean under-estimation of 7 mmol/l.

Sample Characteristics	Measurement Results
Total Concentration = 40.147 mmol/l	
NH ₃ = 100%	31.441 mmol/l
NH ₃ = 67% + NH ₄ ⁺ = 33%	28.316 mmol/l
NH ₃ = 50% + NH ₄ ⁺ = 50%	31.115 mmol/l
NH ₃ = 33% + NH ₄ ⁺ = 67%	34.153 mmol/l
NH ₄ ⁺ = 100%	37.187 mmol/l

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FIGURE LEGENDS

Figure 1: Puncture of the anterior chamber with a 30 gauge needle, pH meter probe in place in the anterior chamber.

Figure 2: Graph of the evolution of pH over 5 minutes. The curve presents 2 successive increases of pH with an interceding plateau.

pH = pH

Temps (mn) = Time (minutes)

Déb NH₃ = Beginning of ammonium hydroxide exposure

Fin NH₃ = Conclusion of ammonium hydroxide exposure

PCA = Anterior chamber puncture total measured ammonium hydroxide concentration

pH th. = Theoretical pH

Figure 3: Graph of the pH evolution over 10 minutes. The curve presents 2 successive increases with an interceding plateau. The increases are less clear because of the scale of the abscissa. There is a peak at about 6 minutes.

[Rest of Legends same as in Figure 2.]

Figure 4: Graph of the pH evolution over 30 minutes. The successive increases of pH have interceding plateaus which partially disappear because of the scale of the abscissa. There is a pH peak at about 6 minutes, preceding an exponential decrease.

[Rest of Legends same as in Figure 2.]